DEVELOPMENT OF A MOUSE MODEL SYSTEM FOR GENETIC SUSCEPTIBILITY AND ITS RELATIONSHIP TO IN VIVO LUNG CARCINOGENESIS

CTR Contract #114
MA Contract 2222

CONTRACT RENEWAL PROPOSAL
FOR THE PERIOD
July 1, 1975 - June 30, 1976

February 12, 1975

Vincent L. Ruwet
Vice President, Contracts and Administration

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DEVELOPMENT OF A MOUSE MODEL SYSTEM FOR GENETIC SUSCEPTIBILITY AND ITS RELATIONSHIP TO IN VIVO LUNG CARCINOGENESIS

It has been long evident that genetic susceptibility is an inherent factor in the incidence of many types of cancer. The human familial incidences of breast and colonic cancer, the predominance of leukemia in a segregant population, and other similar analogies make this a reasonable hypothesis.

Inbred mouse chemical carcinogenesis models, dependent upon subcutaneous chemical carcinogenesis and more recently intratracheally introduced chemical carcinogenesis studies have reinforced the human genetic susceptibility data.

The current "Mouse Model System" contract has investigated and developed the mouse system to four ends. First, to define the appropriate strains of mice to investigate cigarette smoke inhalation effects. Secondly, the program is designed to further define susceptibility to lung cancer. Thirdly, the models developed will be used to attempt to define in a susceptible animal the element risk from exposure to pure chemicals found in tobacco smoke given at different doses. Lastly, long term immunocompetency studies following treatment with chemical carcinogens in susceptible strains have been undertaken in collaboration with Scripps. The techniques developed in these studies will be integrated in a study to define the effects of chronic cigarette smoke exposure and immunocompetency.

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SUMMARY PROGRESS REPORT

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I. SUMMARY PROGRESS REPORT

This report covers all research projects undertaken at MA for CTR on Contracts 2, 14 and 22 during 1974. Although funding has been under several contracts we have undertaken integrated research in an effort to answer some of the biological mechanisms involved in the initiation and development of cancer. In order to study these mechanisms we have studied various genotypically different mouse strains best suited to a particular mechanism.

The CTR-MA contracts have two basic functions:
First, to define a mouse model system for studying various aspects of carcinogenesis.

Second, to undertake screening of known carcinogens, cigarette smoke condensates and condensate fractions by subcutaneous and pulmonary inoculations of mice.

A. Model Systems

1. Chemical Carcinogenesis

a. SC Route (Trioctanoin Vehicle)

In order to define the mouse model systems, we have undertaken subcutaneous carcinogenesis studies with MCA to determine the relative sensitivity of several mouse strains as quickly as possible, and to compare subcutaneous and intratracheal susceptibility. This MCA-SC study (CTR-4) has been completed. Based on the MCA dose required to produce fifty percent tumors (TuD_{50}) in eight months, the most susceptible strain was the C3H/f, followed by the B6C3F1 hybrid using the C3H/f Mai and the C57BL/6 Cum mice. This hybrid is not available commercially but should be considered as a possible susceptible strain after it is characterized for other carcinogens. The time required for tumor development more closely paralleled that of the C3H parent. All the strains were AHH inducible. The C57BL and C57BL/6 mice were virtually negative for type C RNA gs antigen, while nearly all the C3H/f mice were gs^+. The hybrid mice appear to take on the same gs antigen expression as the C3H/f parent.

b. SC Route (Beeswax:Trioctanoin (B:T) Vehicle)

In order to better understand the relationship of tumor induction with B:T vehicle in subcutaneous tumor induction as used for assay of carcinogenic potential of CSC materials, CTR-19 was undertaken with MCA, BaP and DMBA in C57BL/6, C3H/f, DBA/2 and BC3F1 mice. Although these studies are still in progress it would appear that the use of B:T as a vehicle reduces the tumorigenic potential 16x.. This may represent a difference

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in tumor latency, therefore the final results will be necessary before we will see the effect.

c. MCA-IT Route

We undertook two initial studies an in effort to repeat and expand on Nettesheim's intratracheal administration of MCA and the subsequent development of squamous cell carcinomas (SCC) (CTR-3 and 5). Both studies produced only limited numbers of SCC probably due to the use of sonicated material. Later studies with unsonicated material indicate that the particle size of the carcinogen is significant, not only in the type of tumor which develops, but also the latency period for tumor development is greatly reduced with larger particulate material.

Although few SCC were produced in CTR-3 and 5, significant findings were made in CTR-3. It would appear that the hybrid BC3F1 is more susceptible to lung tumor induction than the C57BL, C57BL/6, or C3H/f mouse. This will be studied in later studies. The histological diagnosis of these tumors were adenocarcinomas and alveolar adenomas with relatively few squamous cell carcinomas. In CTR-5 the genetic determination of AHH inducibility was confirmed and it was found that only those mice which were AHH+ developed tumors in the backcross animals (B6 x B6D2). Both CTR-3 and 5 demonstrated that 500µg MCA is exceedingly toxic and many animals are lost during the treatment period due to pneumonia.

Additional studies were undertaken to study the inoculation schedule and diluent for suspending the MCA (CTR-3A and B). These studies have not been completed, however, it would appear that early tumors, and SCC in particular, do not develop with trioctanoin as a diluent. Mice were also injected on a weekly vs. a biweekly schedule. It would appear that the biweekly schedule may be preferable and gel the better vehicle.

Other studies using the IT procedure with MCA are in progress in an effort to determine the TuD_{50} dose for MCA (CTR-7, 7A and 7B). These studies have not been completed, however, they do demonstrate that the survival rate is much better with 250, 125 and 62.5µg MCA and that tumors do occur with a longer latency period with 125 and 62.5µg MCA, particularly when given 12x and to a lesser degree with 6x.

The IT route of MCA is also being used to study the genetic cross and backcross of C3H/f and DBA/2 (CTR-39). Although these studies are still in progress, early results would indicate that tumors have occured in the AHH+ animals.

d. BaP-IT route

Two studies are in progress to determine if Saffiotti's procedure of combining BaP and Fe_2O_3 would result in the production of SCC in mice. The C3H/f mouse has been given 3 dose levels of BaP (0.6, 1.2, 1.8mg/injection) without Fe_2O_3 or combined with 0.6mg Fe_2O_3. These combinations have been administered 5 and 10 times IT in CTR-43. In CTR-44 the DBA/2 mouse

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has been given only the highest dose of BaP (1.8mg) with and without 0.6mg Fe_2O_3. The DBA/2 mice have been on test for only 21 weeks and have shown no evidence of lung involvement. The C3H/f mice have been on test for 38 weeks and up to 75% of the animals treated 10 times with BaP and Fe_2O_3 have developed squamous lesions and/or squamous cell carcinomas. It appears that mice treated 5x may develop tumors, however the latency period is longer and the incidence will be lower.

e. DEN-IT Route DENA ?

In CTR-18 DEN was given to DBA/2 mice in the hopes of developing SCC, however, very few lung tumors developed, however these animals developed liver carcinomas. This model opens up another important aspect of the IT route in that it demonstrates the respiratory route of chemical carcinogens for the induction of tumors in other parts of the body.

f. Beeswax:Trioctanoin Lung Pellet Carcinogenesis

The dose of carcinogen which can be administered by IT inoculation is of necessity rather small and is subject to the need for multiple injections under anesthetic. The implantation of wax pellets of carcinogen used by Stanton in rats has been adapted to mice in our laboratory. This procedure allows larger doses to be given at one time and, in theory, should require only one injection. There are difficulties with this procedure, however, which were not realized when these studies were undertaken. There is a great difference in the leaching rates of different chemicals. There is of course differences in leaching related to the ratios of beeswax to trioctanoin and to carcinogens which are liquids. There are differences in the leaching rate based on the size of the pellet and on the tissue in which it is situated since carcinogens may be water soluble or fat soluble. In the case of multicomponent materials, as (CSC,) the leaching rate of each component will be different thus instead of having a constant exposure to all carcinogens there is probably a serial exposure to one or more carcinogens at a time.

Several studies are now underway using the lung wax implantation procedure. The nitrosamines are being studied this way since it appeared a safer way of handling these materials than by IT inoculation. DMN has been given to both AHH inducible and non-inducible mice in an effort to determine if AHH induction can be correlated with dimethylase activity in any way. This has been done in CTR-18A.

Since DEN, DBN, PYR and PIP have been shown to produce varying lung pathology in C57BL/6 mice when given IP to newborn mice it was thought the aspects of lung carcinogenesis should be pursued by use of the lung pellet procedure. This has been initiated in CTR-45.

To further evaluate this procedure we have also put on test two dose levels of MCA, BaP, DEN and DMBA in C3H/f, C57BL/6 and DBA/2 mice in CTR-18B.

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2. AHH Inducers

Studies were undertaken using a chemical (TCDD) which induces AHH regardless of genotype (CTR-15, 16, 17). Considerable toxicity was encountered. Both C57BL/6 and DBA/2 mice were tested to determine the effects of TCDD on subcutaneous tumor induction. The results with the TCDD are compatible with the idea that AHH induction (via TCDD) simultaneous with MCA treatment yields more tumors than MCA alone, but the results with dioxane are difficult to assess. Why a 48 hours pretreatment with 0.010ml dioxane should enhance MCA-induced tumorigenesis cannot be explained at this time. The fact that both the low and high TCDD levels, when given 48 hours before MCA, had no effect, yet dioxane was also in these treatments, indicates that whatever the effect of dioxane, it is cancelled, if TCDD is present.

This study has been repeated in DBA/2 mice in an effort to determine the problems with dioxane. Results to date (22nd week) indicate that: a) 3% tumors have been observed in MCA-treated D2 mice, b) about 5% tumors in MCA plus dioxane treated mice and MCA plus TCDD, when given IP, treated mice, and c) 31% tumors when TCDD plus MCA given in same vehicle simultaneously.

3. Immunocompetence

The effects of chemical carcinogens on the immunocompetence of the host is believed to be exceedingly important in the growth of transformed cells. In an immunocompetent host the initially transformed cells will not be allowed to grow and progress into a fatal tumor; while an immunosuppressed host may not be able to provide the degree of immune surveillance to prevent tumor development. We have undertaken studies with known chemical carcinogens given IT to define systems which may prove useful in the study of the effects of smoke inhalation at a later date.

a. Intratracheal Instillation of Carcinogen

Our findings indicate that one intratracheal instillation of 500μg MCA was not sufficient to produce immunosuppression in C3H/f Mai, C57BL/6 Cum and DBA/2 J mice of either sex when responses of spleen lymphocytes were tested in vitro three days after exposure (CTR-6A). The responses were unique to the group of mice tested, in that in one experiment they were suppressed and in another they were not. The kinetics (1, 2 or 3 weeks after exposure) of a single instillation was not determined. We did find that after several IT exposured of 500μg MCA that T-cell response was somewhat depressed and B-cell activity significantly so in C3H/f Mai mice (Demoise, Kouri and Whitmire, 1974).

These animals were, however, still capable of an enhanced spleen cell response when challenged with tumor-transplanted cells. It was also interesting that transplanted tumor cells grew slightly better in the MCA exposed mice and this was attributed to the lowered B-cell activity observed which indirectly suggested a lower level of circulating blocking antibodies.

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Host tissue responses as animal, spleen, lung and thymus weights as well as total peripheral white blood cell counts also were not noticeably altered three days after only one intratracheal instillation of 500μg MCA. Again, the kinetics of this treatment was not determined. However, we did find after repeated weekly instillations that noticeable changes occurred such as a reduction in animal and thymus weights, an increase in lung weight and fluctuations in spleen weights and total numbers of white blood cells among the MCA exposed C3H/f Mai mice.

Comparative effects of repeated instillations of 500μg MCA and 1000μg diethylnitrosamine (DEN) on spleen lymphocyte and host tissue responses were studied in C3H/f Mai mice (CTR-6B). As previously observed, both T and B-cell responses of spleen lymphocytes were depressed by repeated instillations of MCA whereas after two and three instillations of DEN, the B and T-cell responses were enhanced. One instillation of 1000μg DEN, like a single 500μg instillation of MCA, was not sufficient to always produce immunosuppression when measured three days after exposure.

In preliminary studies of tumor transplant growth in DEN treated mice we have found no significant differences in growth rates but tumors transplant more readily in the DEN mice. Four and eight exposures to DEN and four exposured of MCA were sufficient to prolong the survival of allogeneic skin grafts. In general, the most tissue responses as animal, spleen, lung and thymus as well as total peripheral white blood cell counts from the DEN treated mice paralleled the changes observed in the MCA mice but were not as pronounced.

The intratracheal instillation of benzo(a)pyrene (BaP) and Fe_2O_3 dust in C3H/f Mai mice was observed to have altered the response of spleen lymphocytes when they were tested after two, six and eleven exposures (D050). Measurements of spleen cell activities, in general, showed (1) after two exposures, a depression of both T and B-cell responses, (2) after six exposures, a greatly enhanced T and B-cell activity, that was more pronounced in the B-cell population, (3) after eleven exposures, a distinctly depressed T-cell response and a much reduced B-cell activity compared to that observed after six exposures. These fluctuations in levels of competence were attributed to an initial depressive effect exerted by the chemical carcinogen, BaP, followed by an over-riding enhanced spleen cell activity exhibited by the host in response to BaP-transformed lung cells.

b. Subcutaneous Administration of Carcinogen

C3H/f Mai and C57BL/6 Cum mice have been exposed to 3-methylcholanthrene (MCA) in subcutaneous implants of wax pellets and the effect that this treatment has on the relationship between levels of host immunocompetence and tumorigenesis is being evaluated (CTR-46). Studies are now in progress that document the capacity of spleen lymphocytes to react to mitogenic stimulation, to neutralize tumor cells transplanted in vivo and to inhibit their growth in vitro at various times after MCA exposure.

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B. Screening of Carcinogens

1. Subcutaneous Route

Earlier studies with MCA, DMBA and BaP have shown the C3H/f Mai strain to be the most susceptible to subcutaneous carcinogenesis. Based on these studies we undertook the screening of 14 fractions of 1R1 and 1A1 reference cigarette smoke condensates to determine their ability to induce tumors alone or with MCA as a cocarcinogen. In order to give as high doses of these fractions as possible, they were implanted as pellets using 1:1 beeswax:trioctanoin as a vehicle. This vehicle has advantages as well as disadvantages. Although it allows larger doses to be given without toxicity, the latency period for tumor development is extended. MCA (150μg) in trioctanoin induced 100% tumors in 20 weeks: while 150μg in beeswax:trioctanoin has produced only 43% tumors in 80 weeks. When 10μg MCA was given in trioctanoin, 38% tumors developed in 82 weeks; however, no tumors developed in the same period when 10μg was given in beeswax:trioctanoin. When 10μg MCA in trioctanoin was injected directly into the fresh beeswax:trioctanoin pellet, as was done with the CSC fraction, 15% tumors have developed in 82 weeks. Further studies to define the relationship between vehicle, carcinogen dose, and tumor latency are planned (CTR-19) using known carcinogens.

The 1R1 fractions have been on test for 82 weeks. Several tumors have developed with the fraction alone, but not at the site of injection. However, with MCA (10μg) as a cocarcinogen, as high as 75% tumors have been induced with the NCH fraction of 1R1. The 1A1 fractions have also been tested in a similar manner. No tumors have developed with the CSC fraction alone, however, with 10μg MCA we have obtained up to 50% tumors with B1a fractions. At this time, 1A1 appears to be less cocarcinogenic than the 1R1 fractions.

Fifteen whole cigarette smoke condensates were obtained through Dr. Gori at NCI. No tumors have occurred in mice receiving only the condensates. As cocarcinogens with 10μg MCA, tumor incidences range from 0-67%, while 15% tumors have occurred with the 10μg MCA control. The hand suckered CSC and the SEB1I were the most tumorigenetic by this assay system.

2. IT Route (CTR-9A, C, D)

1A1-CSC fractions and 15 whole CSC materials have been given IT to C3H/f mice in three injections and observed up to 64 weeks. Those animals which have been sacrificed have failed to demonstrate lung tumors on gross observation. These studies will be terminated over the next several weeks and histological examinations made. These early studies tend to indicate that lower, non-toxic doses should have been given over longer periods

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and possibly it may be necessary to add Fe_2O_3 or a cocarcinogen in order to produce tumors.

3. Wax Pellet Lung Implantation Assay (CTR-9B)

The use of the B:T implant procedure has two advantages over the IT route - i.e., it is a one time operation and larger doses are not toxic. This technique has been used to implant 8 mg whole CSC material in the lung of C3H/f mice. These mice are due to be taken off test during the next few weeks. If no tumors are found it may prove necessary to add a cocarcinogen as was done in CTR-1 for SC tumorigenesis with CSC material.

4. AHH Induction

IT instillation of CSC 1A1 fractions demonstrates that B_1a, B_1b, N_{MeOH} and N_{NM} are good inducers of pulmonary AHH. The whole 1A1 CSC, the reconstituted CSC and fraction N_{eH}, B_e and WA are weak inducers of pulmonary AHH.

The effects of the 1A1 fraction on BP metabolism in vitro was also tested and it was found that fraction B_1b, B_1a, N_{NM} and N_{MeOH} were potent competitive inhibitors of BP metabolism indicating these fractions contain chemicals which definitely influence pulmonary AHH activity.

The induction kinetics of pulmonary AHH following exposure of C57BL/6 mice to one 1A1 cigarette was shown to be rapidly induced and peak activity observed 6 hours post exposure. Liver AHH was unaffected. Exposure to seven consecutive cigarettes was very similar to that observed with one cigarette.

In vitro studies in conjunction with other contracts in our laboratory have studied the relative levels of AHH activities in various cell lines. Results indicate that certain cell lines can have measurable levels of AHH activity and their transforming potential may be related to this AHH activity. This finding may prove useful in the development of an in vitro carcinogenic screening procedure.

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II SUMMARY PROPOSAL FOR CTR-14

July 1, 1975 - June 30, 1976

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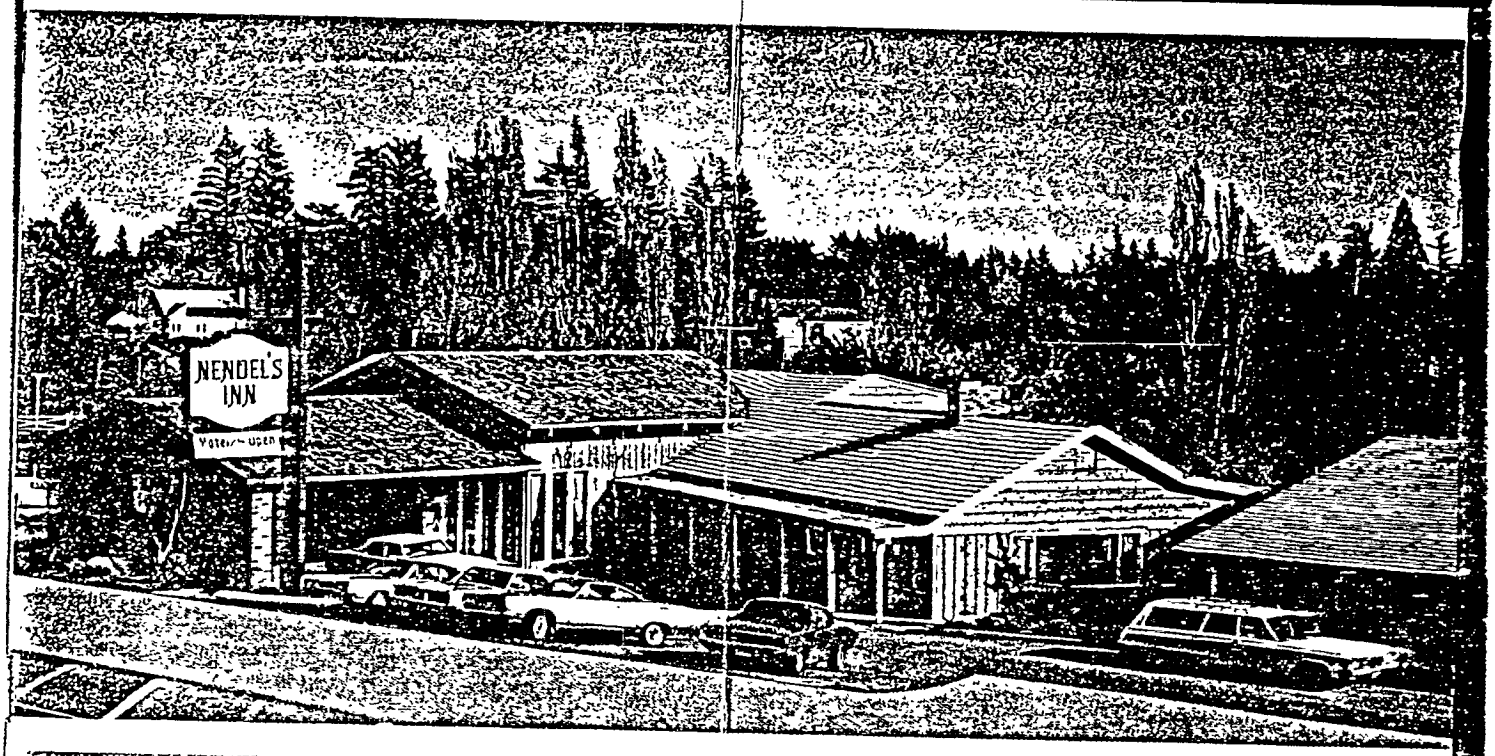

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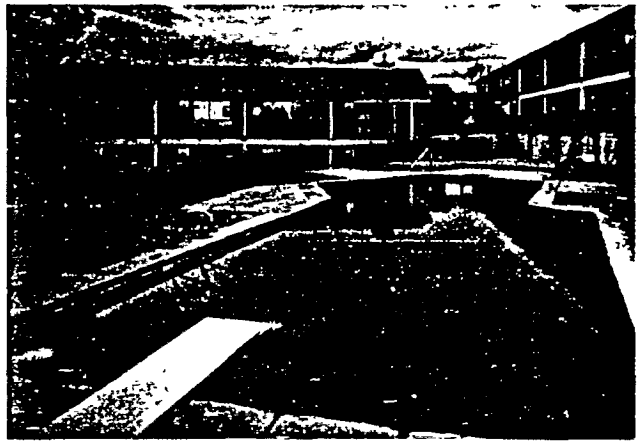

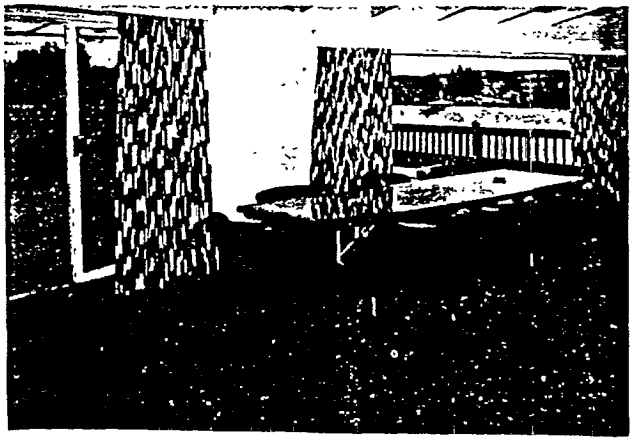

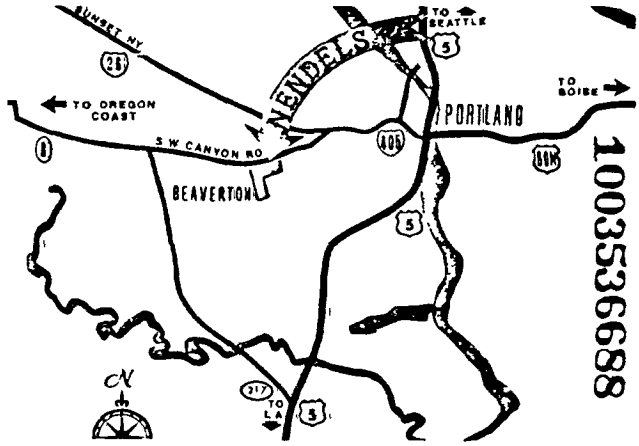

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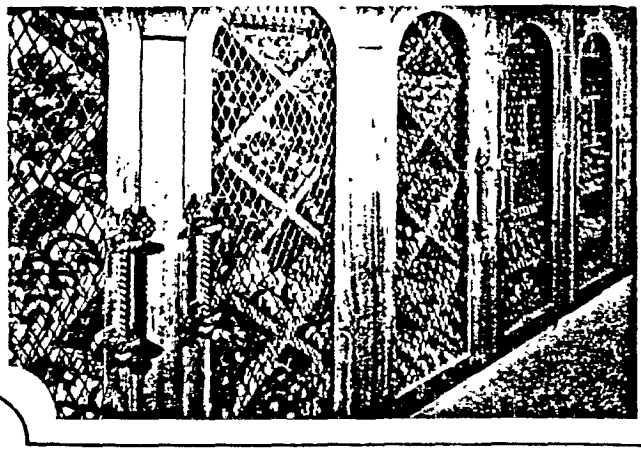

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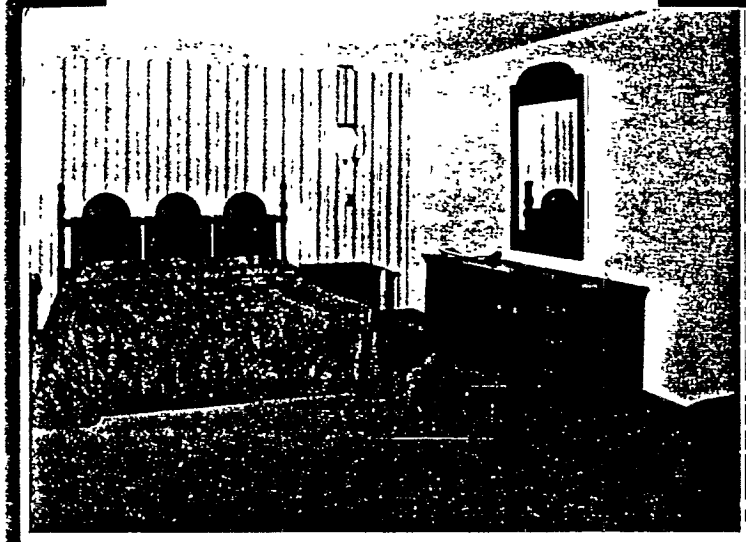

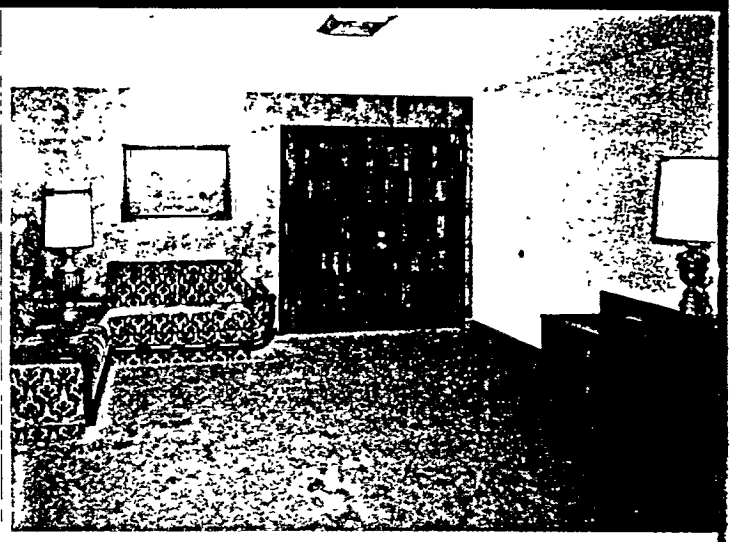

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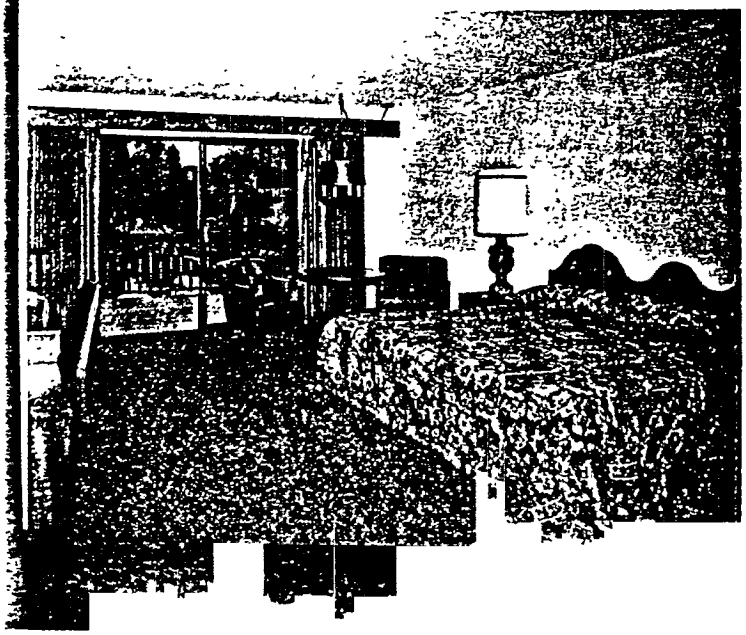

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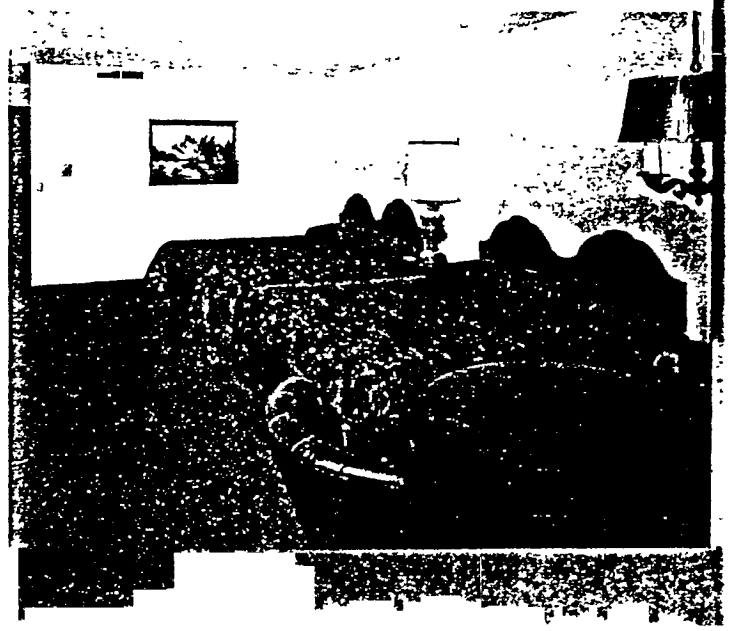

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II. Summary Proposal for Contract Year July 1, 1975 - June 30, 1976 for CTR 14

The proposed studies for the new contract year have been divided into three sections for research and a support section dealing with computer handling of our data. No proposal of the histological work is presented as this represents a classic type service to all the research and bioassay on the CTR programs at MA.

Section A: Chemical Carcinogenesis Assay Systems - C.E.Whitmire, Ph.D.

This contract year will be used to complete the ongoing studies evaluating the MCA & BaP-IT lung carcinogenesis studies. This will demonstrate the best dose and schedule for administration of a known carcinogen and give us leads to be applied in using this system in assay of the carcinogenic potential of CSC materials. We will undertake new studies to determine the early lesions and onset of tumors by serial killing of mice treated with MCA and BaP. These will be useful in evaluating the immunological findings related to the effects of chemical carcinogens found by Dr. Demoise & Dr. Levy. These studies will also establish both the dose and the time of administration of BaP or MCA as a cocarcinogen by the IT route for study with CSC materials or whole smoke.

The use of phorboll esters as a promoter in MCA lung carcinogenesis will be undertaken and if shown useful will be adapted to the assay of CSC materials. The use of Fe_2O_3 in the lung assay of CSC is also a possible approach to be undertaken.

Other studies will evaluate the use of the wax pellet lung implant in the assay of CSC materials. We will also continue our studies of the SC pellet procedure as an assay of CSC materials. These studies have shown promise during the past year and need to be evaluated as to their reproducibility.

Section B: AHH Related Chemical Carcinogenesis, - R.E.Kouri Ph.D.

The completion of the studies proposed for this contract year should go a long way in determining those conditions effecting and/or altering the susceptiblity of pulmonary tissue to chemically-induced cancers. This part of the contract will concentrate on one particular parameter that is known to play a major role in this neoplastic response, the enzyme system aryl hydrocarbon hydroxylase (AHH). The studies will center on the effects of inducers and/or inhibitors of pulmonary AHH (and

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epoxide hydrase) simultaneously with their effects on 3-methylcholanthrene-induced lung carcinomas. The inducing chemical will be the herbicide by-product, TCDD, and the inhibiting chemicals will be 7,8-benzoflavone and trichloropropane oxide. The flavone inhibits "induced" or P-448-mediated AHH, while the oxide inhibits the enzyme system epoxide hydrase. The effects of particular fractions of the IAI condensate will be similarly studied. Certain ongoing studies will probably continue into the new contract year. The studies concerning the effects of whole cigarette smoke on pulmonary AHH will continue in order to observe such parameters as 1) effect of type of smoking machine, 2) effect of diet, 3) effect of type of cigarette, 4) effect of dose of exposure, and 5) effect of AHH genotype. Studies on the role of genetically regulated levels of pulmonary AHH in chemically-induced lung carcinomas will also continue.

Section C: Chemical Carcinogenesis of the Lung - C.F. Demoise, Ph.D.

The studies of the new contract year will include a collaborative study in which the lung tumor induction of MCA is undertaken in Section A to compare with the serial sacrifice of mice for histological finding by Dr. Sass. Animals from the same study will be studied by Dr. Levy at Scripps for immunocompetency and by this section for cellular immunocompetency by various techniques developed during the past year. This collaborative study will lead to better understanding of the relationship of initiation and development of lung tumors by carcinogens entering the lung. These studies will provide information useful in designing future smoke inhalation studies using MCA or BaP as cocarcinogens. The immunological procedures will be applied to future smoke inhalation studies also.

In addition to these studies the effects of immunosuppressive drugs will have on the latency period of MCA induced tumors of the lungs. Studies during the past year with weekly vs. biweekly MCA treatments have demonstrated the biweekly schedulle to give tumors in a shorter period of time. Based on the studies at Scripps the possibility of this shorter latency period is believed to be due, at least in part, to the longer period of immunosuppression induced by MCA.

A new approach will be undertaken to develop a model system for nitrosamine lung carcinogenesis and new studies will be undertaken to develop an intratracheal cocarcinogenesis or two stage carcinogenesis bioassay for CSC materials.

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Section F: Computer Data Handling - Miles J. Haven, M.S.

During the new contract year this section will be involved in increasing the capacity to handle more experiments as well as more observations per animal during the period on test. New summary reports and analysis of data will be undertaken. This will include the generation of graphic plots for better data analysis.

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III PROPOSED STUDIES

SECTION A

CHEMICAL CARCINOGENESIS

C.E. Whitmire, Ph.D.

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SECTION A SCHEDULE December 1, 1974

CTR #	MA #	EXPERIMENT
CTR-3	(W204)	IT-MCA in C57BL, C57BL/6, C3H/Anf, BC3F1.
CTR-3A	(C048)	IT-MCA in C3H/f Mai, gel vs. trioctanoin, weekly vs biweekly injections.
CTR-3B	(C052)	IT-MCA in C57BL/6, gel vs trioctanoin, weekly vs biweekly injections.
CTR-4	(W200)	SC-MCA comparison of C57BL, C57BL/6, C3H/Anf and BC3F1/Cum mice.
CTR-7	(C055)	IT-MCA in C3H/f Mai, 6x weekly vs 12x weekly, 62.5, 125, 250μg.
CTR-7A	(C063)	IT-MCA in C3H/f Mai, 6x vs 12x weekly, 62.5, 125, 250μg.
CTR-7B	(C067)	IT-MCA in C3H/f Anf, 1x, 2x, 4x, 6x/week, 9.38, 18.75, 37.5, 75.0, 150, 300μg doses.
CTR-18A	(C061 & C062)	DMN in B:T pellets, in C3H, C57BL/6, BALB/c, DBA/2, SWR/J mice (AHH + vs -).
CTR-19	(C072)	SC-B:T vs trioctanoin of MCA, BP and DMBA in C3H/f, C57BL/6, BC3F1 and DBA/2 mice.
CTR-21	(C074)	Phorbol ester IT toxicity and AHH inducibility.
CTR-25	(C075)	IT-Phorbol + MCA lung carcinogenesis.
CTR-43	(C058)	IT-BaP + Fe_2O_3, C3H/f mice.
CTR-44	(C068)	IT-BaP + Fe_2O_3, DBA/2 mice.
CTR-45	(C069)	DEN, DBN, PYR, PIP - B:T wax lung implants in C3H/f mice.
CTR-47	1975 - 1976 period	CSC-SC-cocarcinogenesis assay development.
CTR-48		CSC-pellet-cocarcinogenesis assay development.
CTR-50		BaP serial sacrifices for early carcinogenic changes detection.
CTR-51		MCA serial sacrifices for early carcinogenic changes detection.
CTR-52		CSC-IT-Phorbol ester promotor assay development.
CTR-6		Scripps collaborative studies.

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SECTION A SCHEDULE

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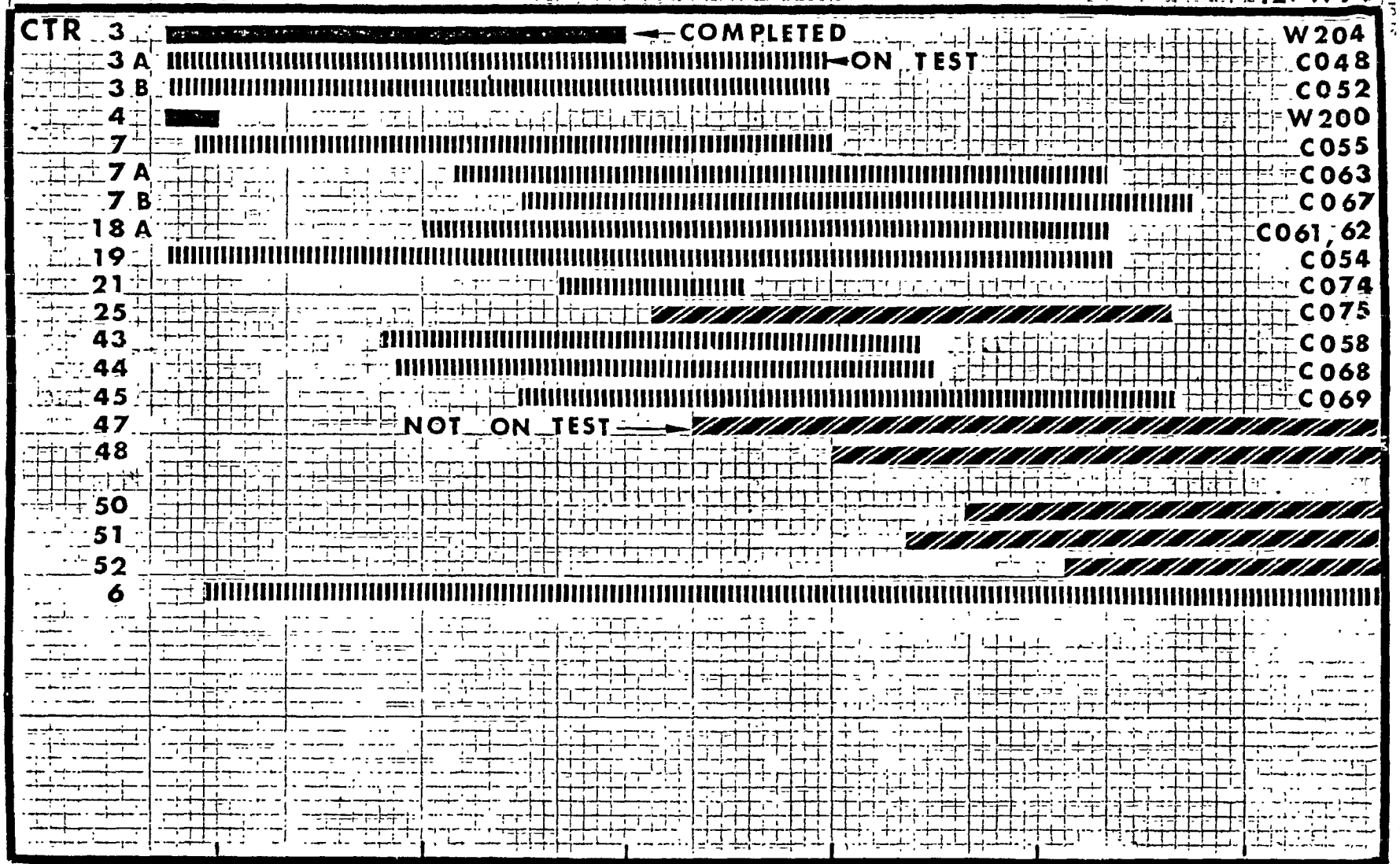

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A. Proposed Studies for the Contract Year

1. Completion of ongoing experiments

a. Intratracheal lung carcinogenesis model

CTR-7, 7A, 7B, These studies are designed to establish the best dose and schedule for inoculation of MCA in the development of the IT lung carcinogenesis model.

CTR-25, This study was designed to develop an animal model system which closely parallels the skin painting assay of carcinogens in which an initiator and promoter are used. It is hoped that phorbol esters will provide a rapid means of producing squamous cell carcinomas in the lungs of mice with low doses of good carcinogens or with higher, non-toxic doses of weak carcinogens.

CTR-43, 44, These studies are concerned with the use of BaP with and without Fe_2O_3 for lung tumor development. They will be completed and new studies probably initiated to further define this assay system.

b. Wax-pellet lung implant carcinogenesis model

CTR-18A and 45, These have been undertaken to develop a system whereby the nitrosamine can be studied in lung carcinogenesis. The pellet implant procedure was undertaken for two reasons: first, it offers a safe way for handling DMN and secondly it allows larger doses of weak carcinogens to be used.

c. Wax-pellet subcutaneous carcinogenesis with known carcinogens

CTR-19, This will be completed to give us a better understanding of this model system which has been shown to be useful for CSC materials in CTR-1.

2. Proposed new studies for the contract year.

During the new contract period this section will be concerned primarily with the development of model assay systems for the assay of tobacco related carcinogens. Efforts will be made to refine the SC cocarcinogenesis assay and to apply this knowledge to the B:T wax pellet lung assay procedure. We will also apply our IT route of inoculation to the assay of CSC materials.

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In addition to these assay procedures, we will continue to define the IT procedures for MCA and BaP by serial sacrifice studies to determine how early the tumors occur and where these tumors arrive.

New collaborative studies with Scripps Institute have been designed to determine the relationship of a single MCA dose ranging from 10μg to 600μg to immunocompetence in the C3H/f mice. These studies will be followed by multiple IT instillations of MCA in which the immunocompetence will be followed and compared to the histopathological findings during the treatment period and during the period when tumors are developing.

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Collaborative Study with Drs. Levy & Lerner at Scripps Clinic and Research Foundation.

Objectives:

To study the immunosuppressive effects of MCA given intratracheally to C3H/f mice.

Procedure:

Mice are given MCA by IT injection at Microbiological Associates, and shipped to Scripps where they are challenged with goat RBC to determine their immunocompetence.

Progress:

#1. During the past year C57BL/6 Cum, C3H/f Mai and DBA/2J mice have been tested for their response to 500μg MCA. These studies showed that immunosuppression occurs in the C57BL/6 and C3H/f but not the DBA/2 mice. These results would indicate the ability to metabolize the MCA may play a role in the immunosuppressive potential since DBA/2 mice are AHH non-inducible.

Proposed Studies:

#1. Studies will be initiated in January 1975 to determine the rellative immunosuppressive effects of various doses of MCA in the C3H/f mice.

#2. A study will be initiated later in the year to follow the immunosuppressive effects of multiple MCA injections. The mice will be followed after the injections have been stopped to determine the effect of tumor development. This study will parallel CTR-51 which will define the histological findings of early lesion development.

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CSC and CSC fraction subcutaneous cocarcinogenesis - assay development.

Objectives:

In previous studies, we have shown a difference in the carcinogenic potential of various whole CSC and fractions can be detected by SC cocarcinogenesis with MCA. There have, however, been differences in the tumor incidences of two IRI CSC tested. This could be due to the differences in these two CSC or in the way they were handled. It is proposed that several whole CSC and several CSC fractions be tested again by this procedure in three consecutive tests to determine the reproducibility of this assay procedure.

Procedure:

Samples would be assayed by the SC route using 10μg MCA and/or a low dose of BaP as a cocarcinogen. The cocarcinogen will be given as previously as a second injection into the pellet area as well as by combining directly with the pelleted CSC material. As a further check on the reproducibility of the assay system, each sample should be assayed at two dilutions to determine the relationship of dose to tumor induction. The B:T ratio used should be 1 part beeswax to 1 and 2 parts trioctanoin.

The C3H/Anf Cum mouse should be used for these studies and they should be terminated at 15 months, since little was gained by holding the previous studies for 18 months.

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Source: https://www.industrydocuments.ucsf.edu/docs/xnyl0000

Cocarcinogenesis using pellet implantation of CSC and CSC fractions in the lung as a carcinogenesis assay procedure.

Objectives:

SC studies with CSC-MCA pellets have demonstrated the necessity of a cocarcinogen to obtain a tumor at the site of injection. This may account for the lack of tumor induction by lung B:T-CSC pellets during the past year. It is suggested that a limited number of CSC and CSC fractions be given with 10μg MCA as pellets to determine if this assay system could be adapted to lung tumor studies with weak and multiple carcinogens present in CSC.

Procedure:

C3H/Anf Cum or B6C3F1 mice will be injected with high, but nontoxic, doses of CSC combined with 10μg MCA or a low dose of BaP in pellets. The mice will be sacrificed at 15 months or when death is imminent and gross and histological examinations made for lung and other tumors. The CSC materials used in CTR-1 provide evidence as to which samples would prove useful for the development of this assay system.

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Source: https://www.industrydocuments.ucsf.edu/docs/xnyl0000

Serial sacrifice for early carcinogenic change due to BaP given IT in C3H/f mice.

Objectives:

In the experiments now on test, we have found numerous BA lesions, and squamous metaplasia which are difficult to classify as squamous cell carcinomas in the absence of metastasis. The only gross indication that lung tumor may have developed in an animal is its general appearance, which may be deceiving. Some animals will become apparently ill with small lung lesions, while others will have massive lung tumors indicating they have had tumors for a considerable period prior to detection. The detection of malignant tumors earlier will of course shorten our experiment time, however, the actual development of tumors is of significance in our immunological studies.

Procedure:

C3H/Anf Cum or B6C3/F1 Cum mice will be given 1.2mg BaP and 0.6mg Fe_2O_3 in 6 biweekly intratracheal injections. Ten mice will be sacrificed at two week intervals starting at the time of the second IT injection. The lungs will be examined grossly for the presence of tumors and fluorescence with a black light. A portion of the lung of 3-5 mice will be minced separately and transplanted subcutaneously to determine at what point in time malignant transformation has occured. This will be correlated with the histological finding of all 10 mice. The spleen and thymuses will be weighed to determine gross changes occurring in the RES system. The spleens of some animals will be used for possible cellular immunological studies based on transplant and histological studies. When frank tumors are grossly detectable in 70% of the mice serially sacrificed for two consecutive periods, the experiment will be terminated and all remaining mice will be sacrificed.

If transplants are successful in the early period during continued treatment with BaP, it may be related to a carry-over of BaP and not necessarily due to the presence of transformed cells. This possibility exists for exceedingly long periods if the yellow appearance of the lungs in our present studies can be considered to be residue BaP. It must also be remembered that the Fe_2O_3 will also be present in the transplanted tissues and may act as a local irritant. The histological findings of the lung tissue will be correlated with the ability to transplant the lung tissue. If the residue BaP does not prove to be a carcinogenic factor, then we may be able to determine at what stage the squamous metaplasia can be considered to be a squamous CA in the absence of metastasis.

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CTR-51 December 1, 1974

Repeat of CTR-50 using MCA to induce lung tumors by IT inoculation.

Objectives:

This study will answer some of the same questions as the BaP experiment. It is felt essential it be done with MCA also since we are using the MCA system for determining the immunological suppressive effect in the Scripp's studies. We will be shipping mice to them at varying intervals after inoculation for immunological studies and these should be correlated with histological findings to make their findings meaningful. Studies will be undertaken in our laboratory to correlate with cellular immunity.

Procedure:

Same as CTR-50 only MCA will be substituted for BaP and no Fe_2O_3 will be used.

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Phorbol ester promoter effects with CSC and CSC fractions given by IT route for lung carcinogenesis assay development.

Objectives:

To apply the well known use of the promoter action of phorbol esters to lung carcinogenesis it is suggested the nontoxic dose established in CTR-21 be combined with CSC and CSC fractions given IT to produce lung tumors. This study will only be undertaken in the event PE proves usefull with MCA (CTR-25).

Procedure:

BC3F1 or B6C3F1 mice will be treated with low doses of CSC at biweekly intervals with phorbol ester being administered on a biweekly schedule. Such a schedule would give phorbol ester in weeks 1, 3, 5, etc. and the CSC in weeks 2, 4, 6 etc. In this way, lung tumors may be produced in much the same way as skin tumors are produced in the standard in vivo bioassay now in use.

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SECTION B

AHH RELATED CHEMICAL CARCINOGENESIS

R.E. Kouri, Ph.D.

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Source: https://www.industrydocuments.ucsf.edu/docs/xnyl0000

SECTION B SCHEDULE December 1, 1974

CTR #	MA #	Experiment
CTR-5		IT-MCA in C57BL/6, DBA/2 and B6D2F1
CTR-10	K-16	Effects of 1A1 CSC fractions on AHH activity.
CTR-11	K-17,39	Kinetics of pulmonary AHH activity with 1A1 smoke exposure.
CTR-15,17	K-29,31	TCDD effects of MCA-SC carcinogenesis.
CTR-21	K-47	Phorbol ester pulmonary AHH induction (mouse studies in Section A, AHH assay in Section B)
CTR-23	K-41	SC-MCA in C3H/f x DBA/2
CTR-24,39	K-44	IT-MCA in C3H/f x DBA/2
CTR-36-37		In vitro chemical carcinogenesis with C3H 3T 10 1/2 cells.
CTR-40	K-42	SC-MCA TCDD studies in DBA/2 mice (repeat of CTR 15,17).
CTR-20	K-46	DMN effect on pulmonary AHH induction.
CTR-58		Effects of TCDD on pulmonary AHH activity
CTR-59		Effects of TCDD on lung cancer induced by MCA
CTR-60		Effects of 7,8-benzoflavone on pulmonary AHH activity induced by MCA
CTR-61		Effect of 7,8-benzoflavone on pulmonary tumors induced by MCA
CTR-62		Effect of triochloro-propane oxide (TCPO) on pulmonary AHH and epoxide hydrase (EH) activity
CTR-63		Effects of TCPO on lung tumorigenesis induced by MCA
CTR-64		To observe the role of particular fractions of the 1A1 condensate on lung tumors induced by MCA

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SECTION B SCHEDULE

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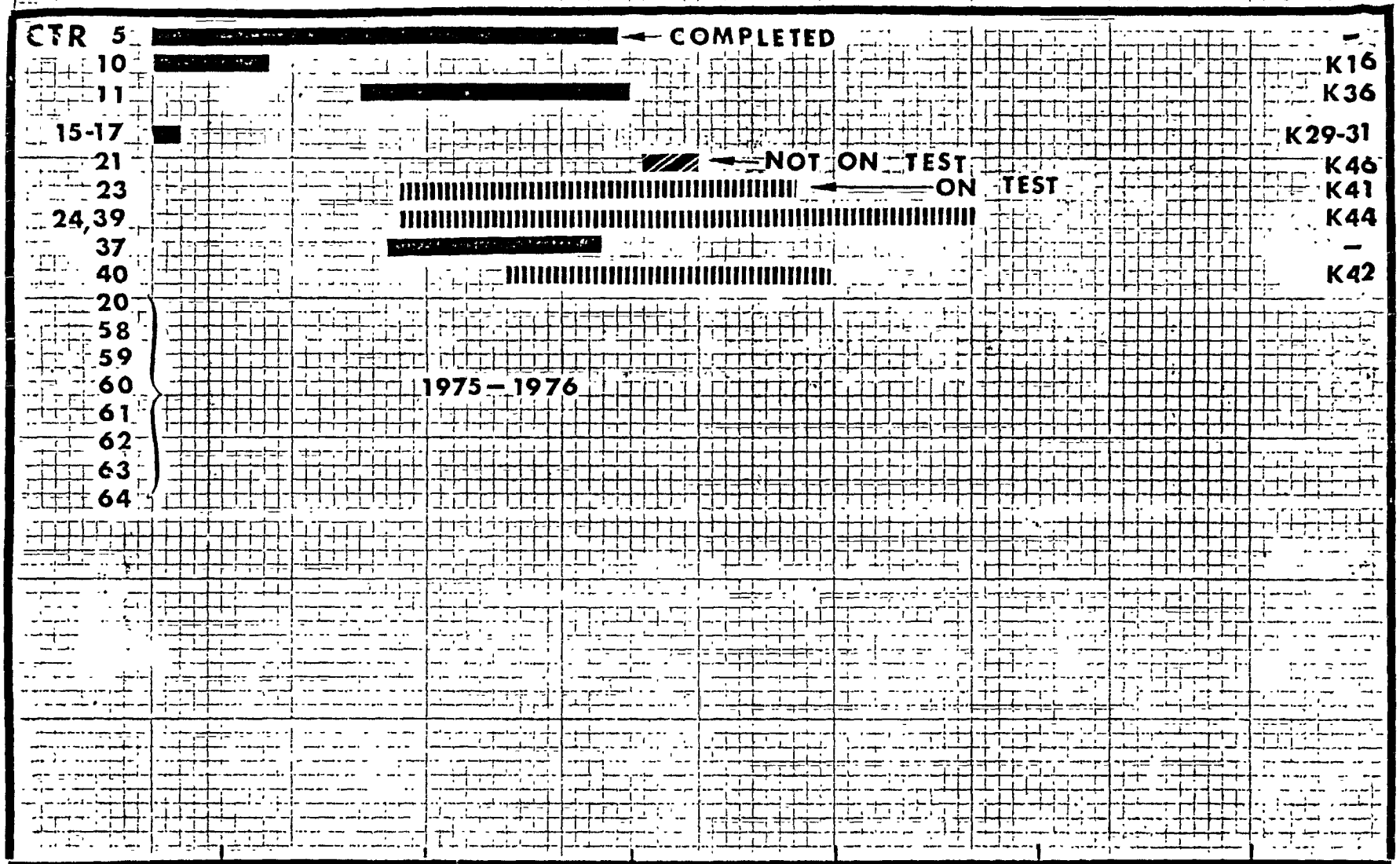

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A. Proposed Studies for the Contract Year.

1. Completion of ongoing experiments.

a. Effects of various substances on lung AHH induction.

CTR-11 Kinetics of pulmonary AHH activity with 1A1 smoke exposure.

CTR-20 DMN effect on pulmonary AHH induction.

CTR-21 Phorbol ester pulmonary AHH induction (Mouse studies in Section A, AHH assay in Section B).

b. Intratracheal lung carcinogenesis.

CTR-24 IT-MCA in C3H/f x DBA/2

c. Subcutaneous Carcinogenesis

CTR-23 SC-MCA in C3H/f x DBA/2

d. Effects of AHH inducers on chemical carcinogenesis.

CTR-40 SC-MCA TCDD studies in DBA/2 mice (Repeat of CTR 15, 17)

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CTR-20

Effect of Dimethylnitrosamine (DMN) on Pulmonary AHH Levels

Objectives:

Observe the effects of DMN or MCA pretreatment on ability of inducible and noninducible mice to metabolize these carcinogens.

Background:

DMN is a potent chemical carcinogen which is found in small quantities in cigarette smoke and may be formed in situ by nonenzymatic reactions involving inorganic nitrite and amines. Results suggest that DMN must be metabolically activated to its carcinogenic form and the enzyme causing this activation is DMN-demethylase. The presence of this enzyme bears a mirror-image relationship to the presence of the AHH enzymes. Compounds which induce high levels of AHH seem to be potent repressors of DMN-demethylase activity. Thus, animals which are noninducible for AHH activity should be more sensitive to DMN-induced tumors. This conclusion is based on the fact that (1) DMN may be an inducer of AHH activity, (2) DMN would repress DMN-demethylase activity, in inducible animals, (3) DMN would have no effect on DMN-demethylase activity in noninducible animals. The in vivo study designed to test this hypothesis is in CTR-31. Certain biochemical tests should predate this test and these are given in this protocol. Some of these studies may involve collaboration with Dr. J. Arcos, Tulane University, New Orleans, Louisiana.

Procedure:

a. Groups *(3 mice per group)

Treatment	Strains B6	Strains D2
DMN	*3	3
MCA	3	3
DMN + MCA (given simultaneously)	3	3
DMN + MCA (DMN 24 hrs. previous to MCA)	3	3
DMN + MCA (MCA 24 hrs. previous to DMN)	3	3
Untreated	3	3

b. Protocol
(1) Determine if DMN-demethylase activity is measurable in the lungs of B6 and D2 mice. If so, then all assays will follow intratracheal administration of the chemicals. If not, then all assays will follow intraperitoneal administration and will be performed on hepatic rather than pulmonary tissue.

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(2) Determine dose effects of DMN on demethylase and AHH activity. Use dose of DMN giving maximum induction of AHH in the B6 animals.

Progress:

The initiation of this study is awaiting some recent data from the laboratories of Drs. J. Arcos and M. Arcos. These experimentors are trying to assay for DMN-demethylase activity in lung tissue and the techniques are presently being improved.

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CTR-21

Effects of Promotors on Pulmonary AHH Activity

Objectives:

To establish whether phorbol ester alters levels of pulmonary AHH activity.

Background:

Promotors are substances with the property of increasing the carcinogenic effect of other compounds. Recent data suggest that the mechanism of action of promotors may be related to the fact that all of these compounds are very good inhibitors of DNA repair. Compounds such as phorbol esters, vitamin A, or particular steroid hormones are examples of chemicals which inhibit DNA repair and are potent promotors of chemically-induced cancers. Since both vitamin A and steroid hormones directly influence AHH activity, there may be a relationship between promoting activity, repair inhibition, and AHH enzyme levels.

Procedures:

a. Groups

Treatment	Time (Days) 1	7	14	21
PE	3	3	3	3
PE + MCA	3	3	3	3
MCA	3	3	3	3
Control	3			

*Number of animals.

b. Protocol
(1) Determine a dose of PE that when given IT (in 0.02 ml) will kill about 10% of the animals in 21 days (LD_{10-21}). The dose will probably be about 0.01μg per mouse.
(2) At this dose level, give PE twice a week and observe constitutive and MCA induced AHH levels at the times indicated.
(3) Repeat for both strains of mice.

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Progress:

Determination of the LD(10-21) is presently being determined; the available results are shown in CTR-21. The AHH studies should begin by February-March, 1975.

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2. New experiments.

Introduction: Recent results using our SC mouse model system for carcinogenesis of MCA has demonstrated that not only is susceptibility to chemical carcinogenesis in inbred strains of mice regulated to a major degree by the genetic regulated levels of AHH, but also, this genetic regulation can be altered somewhat by the use of other chemicals which can alter AHH regardless of genotype (e.g. TCDD) Of particular importance, is the fact that the treatment schedules for TCDD and MCA play a major role in this altered sensitivity. Co-treatment of MCA and TCDD enhances the tumor response in MCA-non-responsive mice (e.g. DBA/2), however a 48 hour pretreatment with TCDD does not effect MCA tumorigenesis. This is somewhat surprising in that a 48 hour pretreatment will mean that there is maximal AHH activity at the time of MCA administration and one may think that this schedule should yield a maximum tumorigenic response. The fact that this was not the case suggests that AHH activity by itself is not the determining factor, but rather <u>when</u> the activity is increased is important.

Our rather simplistic working hypothesis explaining these results is that there are definite differences between the kinetics of induction of the epoxide-forming enzymes (e.g. AHH) and the epoxide-degrading enzymes (e.g. epoxide hydrase (EH)). During the first 24 hours, the levels of the two enzymes are comparable. Thus, we feel that during the 1st 24 hours, the epoxide has a longer effective lifetime and it is during this time that the neoplastic process is initiated. This hypothesis is very difficult to prove, or disprove, using the SC tumorigenesis system because AHH activity cannot be effectively quantitated in the tissue in which the tumor originates (e.g. connective tissue).

However, with the advent of the carcinogenesis system utilizing the IT administration of MCA to induce pulmonary carcinomas, this hypothesis can be tested. The lung provides the unique situation of being able to quantitate the enzymatic responses in the same tissue in which the tumor eventually occurs. Thus, observing the effects of inducers and/or inhibitors of pulmonary AHH simultaneously with their effects on MCA-induced carcinomas should allow for the determinations of these conditions that maximize susceptibility to lung cancer. These are the conditions that must be utilized to ascertain the relative carcinogenic as well as co-carcinogenic effects of whole cigarette smoke. Cigarette smoke may function as a co-carcinogen by inducing AHH in a manner analogous to TCDD. Thus, it may not be the actual amount of AHH

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activity that is induced by cigarette smoke, but the relative time between induction and exposure to other known chemical carcinogen pollutants that is important to cancer production. Likewise, cigarette smoke may be a complete carcinogen, but, because of its fairly weak nature, almost perfect conditions must be present before its carcinogenic activity can be observed. Pre-, post- or co-treatment with an inducer of AHH may give these conditions. The experiments outlined in the following section should go a long way in determining these conditions.

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Effects of inducers of AHH.

CTR-58 Effects of TCDD on pulmonary AHH activity.

Objective: Preliminary results from CTR-40 suggest that the inherent susceptibility to MCA carcinogenesis may be altered via the use of a chemical which induces AHH in strains of mice nonresponsive to MCA alone. Thus, the potential susceptibility of an individual may be determined by not only its genetic mechanism regulating AHH, but also the exposure to other chemicals whose effect may mitigate this genetic regulation. These studies (CTR-58 & 59) are designed to observe the effects of TCDD on the susceptibility of AHH-responsive and non-responsive mice to MCA-induced lung carcinomas.

Materials:

(1) Mice

(a) C3H/Anf Cum, ♀, 8-10 weeks old
(b) C57BL/6 Cum, ♀, 8-10 weeks old
(c) DBA/2 Cum, ♀, 8-10 weeks old

(2) TCDD in dioxane at concentrations of 100nM, 10nM, 1nM, 0.1nM.

Method:

(1) IT instill 0.02ml to 5 mice of each strain

(2) At 24 hours, kill by cervical dislocation, remove livers and lungs and assay for AHH activity.

(3) For kinetics, determine minimal dose of TCDD capable of inducing pulmonary, but not hepatic, AHH and observe amount of enzyme at 2, 4, 6, 12, 24, 48, 72 and 96 hours post treatment.

(4) Epoxide hydrase activity may also be monitored in these same tissues.

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CTR-59 Effects of TCDD on lung cancer induced by MCA.

Objective: To determine the role of TCDD-effected levels of pulmonary AHH in MCA induced pulmonary carcinomas. These studies are based on the findings in CTR-40 and CTR-58.

Materials:

(1) Mice

(a) C57Bl/6 Cum (or C3H/Anf Cum), ♀, 8-10 weeks old

(b) DBA/2 Cum, ♀, 8-10 weeks old

(2) TCDD (as dose level determined in CTR-58)

(3) MCA at 250μg/0.02ml 0.2% gelatin in sterile saline.

(4) TCDD and MCA in same vehicle.

Groups:

(1) For C57Bl/6 mice (or C3H)

Treatment:

	-2 days	0 days	# mice
1.	Diox	Trioc	50
2.	TCDD	Trioc	50
3.	None	MCA	100
4.	Diox	MCA	100
5.	TCDD + MCA	None	100
6.	TCDD	MCA	100
7.	TCDD + MCA (same vehicle)	None	100

(2) Repeat groups for DBA/2 mice.

Procedures:

(1) Follow protocol, and give MCA 1, 2 or 3 times, (depending on results of ongoing experiments).

(2) At 2 months post treatment take 5 animals per group off test and observe macro and microscopically for lung tumors.

(3) When tumors found, take off remaining animals and observe for pulmonary tumors.

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Effects of inhibitors of AHH activity.

CTR-60 Effects of 7,8-benzoflavone on pulmonary AHH activity induced by MCA.

Objective: 7,8-BF is a potent inhibitor of "induced" (or P-448)-mediated AHH activity. What would be the effects of this compound on pulmonary AHH? Under what conditions are maximal inhibitions observed? The use of this compound should decrease the amount of intracellular epoxide, the hypothetical ultimate carcinogen, but competitively inhibiting the metabolism of MCA.

Materials:

(1) Mice: C3H/Anf Cum, ♀, 8-10 weeks old

(2) 7,8-BF dissolved in 0.2% gelatin in sterile saline, or dissolved in trioctanoin.

(3) MCA (200μg/0.02ml gelatin solution)

Procedures:

(1) IT instill 200μg MCA plus various amounts of 7,8-BF in the same vehicle (total volume 0.02ml).

(2) Doses of 7,8-BF should be 0, 50, 100, 200 and 500μg.

(3) At 24 hours after treatment remove livers and lungs and assay for AHH activity.

(4) At dose level, giving maximum inhibition determine the kinetics of this inhibition.

1003536217

Source: https://www.industrydocuments.ucsf.edu/docs/xnyl0000

CTR-61 Effect of 7,8-BF on pulmonary tumors induced by MCA.

Objective: To observe the biological consequences of the results observed in CTR-60. The conditions that allow for a decreased amount of intracellular epoxide should definitely influence the relative carcinogenicity of that particular dose of MCA.

Materials:

(1) Mice: C3H/Anf Cum, ♀, 8-10 weeks old

(2) 7,8-BF at concentration determined by CTR-60.

(3) MCA at 200μg/0.02ml gelatin solution.

Groups:

Treatment	Animals
Trioc or Gel	50
BF	100
MCA	100
BF + MCA	100

Procedures:

(1) Follow treatment schedule determined in CTR-60 to yield maximum pulmonary AHH inhibition.

(2) At 2 months post-treatment, take 5 animals off test and observe macro- and microscopically for lung tumors.

(3) Repeat every month until tumors observed.

(4) Record incidence as well as latency of tumor formation.

1003536718

Source: https://www.industrydocuments.ucsf.edu/docs/xnyl0000

CTR-62 Effect of trichloro-propane oxide (TCPO) on pulmonary AHH and epoxide hydrase (EH) activity.

Objective: TCPO is a potent inhibitor of EH activity. This enzyme degrades epoxides to a noncarcinogenic end product, the dihydrodiols, and thus can affect the instantaneous level of this carcinogenic intermediate. Can this chemical affect AHH activity and EH activity *in vivo*?

Materials:

(1) Mice: C3H/Amf Cum, ♀, 8-10 weeks old

(2) MCA at 200μg/0.02ml gelatin solution.

(3) TCPO at various concentrations and suspended in the MCA vehicle.

Procedures:

(1) Determine relative toxicity of TCPO when given IT. (It is a very toxic compound.)

(2) Determine level of TCPO that when given with 200μg MCA, will alter AHH activity and inhibit EH activity at 24 hours post treatment.

(3) Determine the kinetics of this inhibition.

(4) May have to look at the spectrum of metabolites using thin layer chromatography, (in collaboration with Drs. Wang and Rasmussen) in order to be certain that the actual level of epoxide has increased, and not that the level of another metabolite has increased.

100353719

Source: https://www.industrydocuments.ucsf.edu/docs/xnyl0000

CTR-63 Effects of TCPO on lung tumorigenesis induced by MCA.

Objective: To observe the tumorigenic consequences of CTR-61. The use of TCPO should increase the level of epoxide by decreasing the rate of degradation to the non-carcinogenic dihydrodiols. This should enhance the tumorigenic effects of that particular dose of MCA.

Materials and Procedures are similar to that described in CTR-60.

1003536720

Source: https://www.industrydocuments.ucsf.edu/docs/xnyl0000

Effect of fractions derived from the 1A1 cigarette smoke condensate.

CTR-64 To observe the role of particular fractions of the 1A1 condensate on lung tumors induced by MCA.

Objective: Particular fractions of the 1A1 condensate can interrupt AHH activity in vivo. Fractions B_1a (representing 1.0% by weight of the whole condensate) is a potent inducer of pulmonary AHH activity. Fraction SA_W (representing $\approx$ 40% of the total condensate) is a good inhibitor of pulmonary AHH activity. What are the effects of these two fractions on lung tumorigenesis induced by MCA?

Materials:

(1) Mice: C3H/Anf Cum, ♀, 8-10 weeks old

(2) MCA (200μg/0.02ml gelatin solution)

(3) B_1a and SA_W fractions as same concentration ($\approx$ 1000μg/ml) in this same vehicle.

Procedures:

(1) Determine if the B_1a or SA_W fraction will inhibit pulmonary AHH activity when given in same vehicle with MCA. Although the B_1a fraction induces AHH activity it may also inhibit induction by MCA by competative means.

(2) Studies into the effects of these fractions on MCA carcinogenesis will proceed based on these preliminary findings.

1003536721

Source: https://www.industrydocuments.ucsf.edu/docs/xnyl0000

SECTION C

CHEMICAL CARCINOGENESIS

C.F. Demoise, Ph.D.

10035367722

Source: https://www.industrydocuments.ucsf.edu/docs/xnyl0000

SECTION C SCHEDULE December 1, 1974

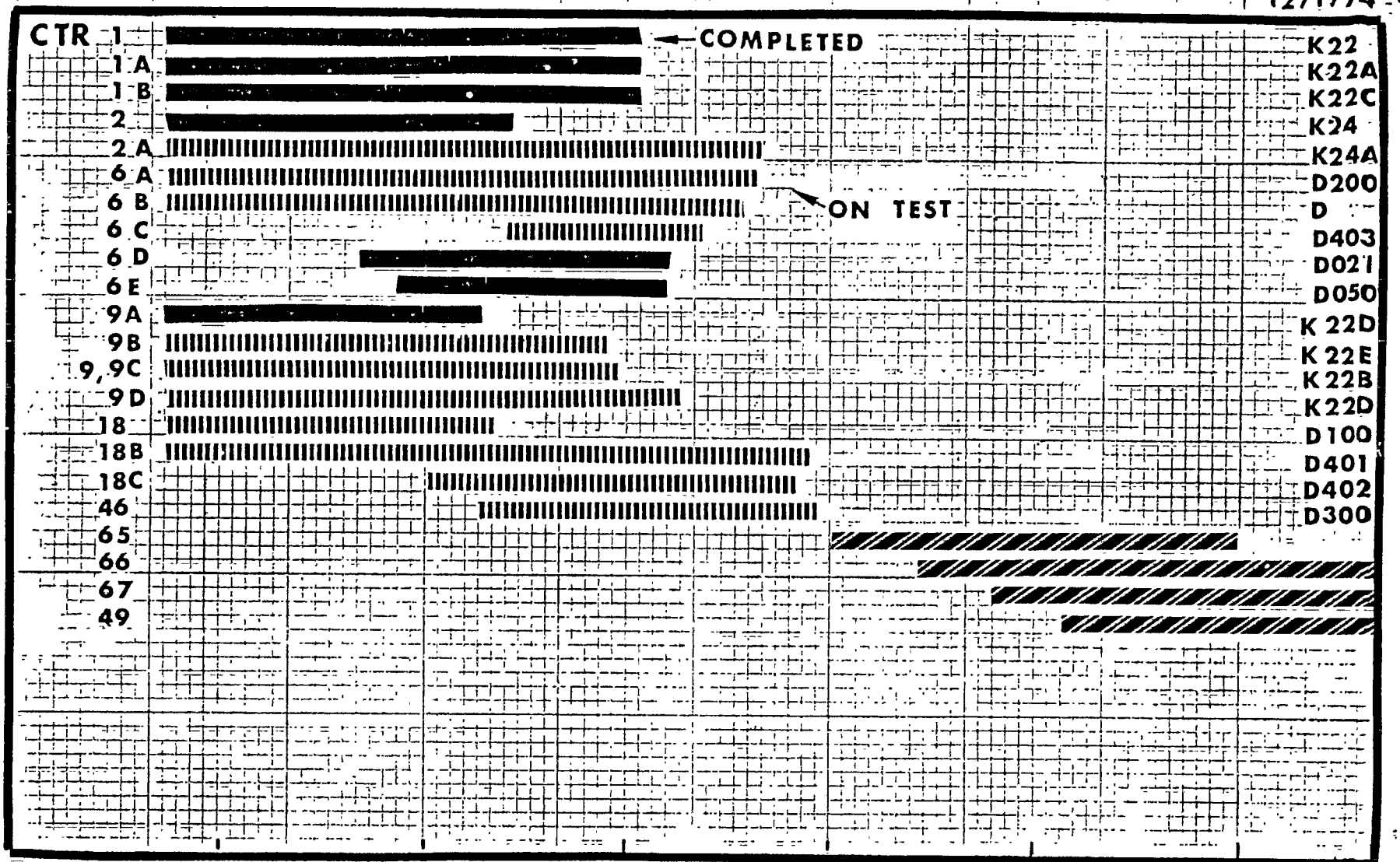
CTR #	MA #	Experiment
CTR-1	(K22)	SC-B:T pellets IRI fractions - MCA cocarcinogenesis.
CTR-1A	(K22A)	SC-B:T pellets IAI fractions - MCA cocarcinogenesis.
CTR-1B	(K22C)	SC-B:T pellets, 15 whole CSC - MCA cocarcinogenesis.
CTR-2	(K24)	IP - Nitrosamine carcinogenesis in C57BL/6 NB mice.
CTR-2A	(K24A)	Repeat of CTR-2.
CTR-6A	(D200)	Immunocompetence - IT-MCA in C3H/f, C57BL/6, and DBA/2 mice.
CTR-6B	(Dseries)	Immunocompetence - It-MCA and DEN in C3H/f mice.
CTR-6C	(D403)	Direct implantation of tumor cells and carcinogens in lung.
CTR-6D	(D021)	Immunocompetence - IT-Fe_2O_3 in C3H/f, C57BL/6 and DBA/2 mice.
CTR-9A	(K22D)	IT-IAI fractions.
CTR-9B	(K22E)	B:T lung implants of 15 CSC samples.
CTR-9 & CTR-9C	(K22B)	IT-15 CSC samples.
CTR-9D	(K22D)	IT-IAI fractions.
CTR-18	(D100)	IT-DEN implant in DBA mice.
CTR-18B	(D401)	B:T lung implants of MCA, BaP, DEN and DMBA in C3H/f, C57BL/6 and DBA/2 mice.
CTR-18C	(D402)	BaP - B:T lung pellet leaching rates.
CTR-46	(D300)	Cell-mediated immune response in mice after SC implantation of wax pellets - MCA.
CTR-65		To determine if immunosuppression plays a role in MCA lung tumor development.
CTR-66		To follow the immunocompetence of C3H/f mice treated by intratracheal instillation of MCA.
CTR-67		To develop an assay for lung carcinogenesis with the nitrosamine compounds.
CTR-49		CSC-IT cocarcinogenesis and/or two-stage carcinogenesis assay system.

100353723

Source: https://www.industrydocuments.ucsf.edu/docs/xnyl0000

SECTION C SCHEDULE

12/1/74



1003536724

A. New Studies for This Contract Year

1. Bioassay for lung cancer

Preliminary studies carried out during the past year have demonstrated that whole CSC or CSC fractions will not produce lung tumors after 3 injections at a nontoxic dose. It has been shown that SC pellets of CSC or fractions only produce tumors when combined with MCA co-carcinogenesis. It is suggested that the same phenomenon may be true for lung carcinogenesis. Studies are now underway to determine the TuD_{50} via IT inoculation in C3H/f mice for MCA. By combining a subtumorigenic dose of MCA with a nontoxic dose of CSC it may be possible to develop an assay for the lung cancer potential of various tobacco or smog associated chemicals. Such an assay would be more meaningful than the classic skin painting assay now used. It would not only allow the development of a lung tumor assay system but would evaluate this system as an assay procedure for carcinogens normally entering the body by the respiratory tract, but producing tumors elsewhere in the body. This has recently been shown in our laboratory by the IT inoculation of DEN and the development of liver tumors.

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Source: https://www.industrydocuments.ucsf.edu/docs/xnyl0000

Objective:

CSC-IT cocarcinogenesis and/or two-stage carcinogenesis assay system.

Procedure:

C3H/Anf Cum and/or B6C3F1 mice will be injected at biweekly intervals for 6-12 injections with subtoxic doses of CSC and CSC fractions combined with 10μg MCA. Mice will be observed for evidence off imminent death before they are sacrificed for gross and histopathological examination.

The use of Fe_2O_3 in combination with the CSC material may prove useful and should be considered since it appears useful in the BaP-Fe_2O_3 experiments.

The vehicle used for suspending the CSC may also be important as shown with the MCA studies; therefore, this aspect will have to be considered in designing these studies.

The use of a promoter as TPA may also prove useful and will be studied if shown useful with low doses of MCA.

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Source: https://www.industrydocuments.ucsf.edu/docs/xnyl0000

2. New Studies to Develop a Model System for Assay of Nitrosamine Carcinogenesis in Mouse Lung Tissues.

During the past several years we have realized the urgent need for consideration of the nitrosamines in lung carcinogenesis. During this period we have been unable to equate the presence of the demethylases to nitrosamine carcinogenesis as we have the AHH system with PAH carcinogenesis. This type of study awaits further definition of this area by Dr. Arcos.

During the new contract year we propose to try a new approach to the study of nitrosamine lung carcinogenesis as outlined briefly in the proposed study.

1003536227

Source: https://www.industrydocuments.ucsf.edu/docs/xnyl0000

CTR-67 December 20, 1974

Objective:

To develop an assay for lung carcinogenesis with the nitrosamine compounds.

Background:

The use of IP inoculations of newborn C57BL/6 mice has demonstrated that lung adenomas can be produced in 6-12 months in a limited number of mice. The use of DEN in IT studies failed to produce lung tumors but rather produced liver tumors. Studies are under way using the lung pellet implant procedure with a number of nitrosamines but the assessment of this assay system will require possibly another year.

We propose that a new approach be taken which will insure the assay of nitrosamines for lung tissue will not result in tumors in other tissues by the procedure outlined below.

Procedure:

Mice of several genotypically different backgrounds as the C3H/f, C57BL/6, DBA/2 and possibly others will be anesthetized and the lungs will be infused with various nitrosamine compounds suspended in tissue culture medium believed to be involved in lung carcinogenesis. The lungs will be removed from the animal and held 4 hours in tissue culture medium under the best conditions to allow survival of the cells. The tissue will then be minced and allow time for dialysis of the nitrosamines from the tissues overnight. After this period the tissue will be transplanted either subcutaneously or IP in newborn mice and observed for tumor development. If tumors develop, histological diagnosis of the tumor type will be done to determine if we have, in fact, produced adenomas, adenomocarcinomas or squamous cell carcinomas.

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Source: https://www.industrydocuments.ucsf.edu/docs/xnyl0000

3. New Studies Related to the Immunological Aspects of Chemical Carcinogenesis for the New Contract Year

The studies for the past year have shown some evidence of the suppression effects of chemical carcinogens on the immune competence of the mouse. However, these results have not been as clear cut as we would have hoped them to be. The results of Drs. Richard Levy and Richard Lerner have demonstrated there is probably an association of this ability of the genotypically different strains to metabolize methylcholanthrene (AHH inducibility). Their studies and those carried out in our laboratory by Dr. Demoise have also demonstrated the difficulties in studying cellular immunity in the mouse due to the wide variations in responses after a single intratracheal administration of MCA.

In the new contract year we propose to continue these studies, however to limit this effort and concentrate on an area where we have some evidence of more consistent findings. Our studies have indicated that T and B-cell responses may differ after multiple MCA treatments and during the period when the transformed cells develop into tumors. We feel if we undertake a definitive study with MCA and/or BaP which is paralleled with histological observations and the response to goat RBC by Dr. Levy that we may be able to better define why preliminary results indicate that 6 IT treatments over 12 weeks is superior to 6 treatments over a period of 6 weeks. Another aspect which we feel must be covered by such a study is to determine the kinetics of immune suppression related to ability of spleen cells to respond and the number of cells available for response at varying times after MCA treatment. This may explain differences in in vitro spleen assays and in vivo immune responses which have occurred.

Another aspect of the part immune competence may play in chemical carcinogenesis will also be investigated by immunosuppressing the host in an attempt to shorten the latency period of lung tumor development and possibly decrease the number of treatments required for carcinogenesis.

10035367 29

Source: https://www.industrydocuments.ucsf.edu/docs/xnyl0000

CTR-65 December 20, 1974

Objective:

To determine if immunosuppression plays a role in MCA lung tumor development.

Background:

Studies carried out under CTR-3 and CTR-7 have demonstrated that the schedule of MCA treatment is possibly more important than the total dose of carcinogen. This may indicate one possible factor at play would be a longer period of immunocompetence with a biweekly schedule vs. a weekly schedule of MCA treatment. If this is true then the period following MCA treatment would be a period when immune competence would be regained and delay tumor development. This also suggests that this hypothesis of immune suppression as a factor in tumor development can be proven if the host is treated with immunosuppressive drugs to allow rapid development of the tumors from cells which were transformed by possibly the first MCA treatment. If we can obtain tumors more rapidly by immunosuppression then one treatment may work as well as multiple carcinogenic exposures.

Procedure:

C3H/f mice will be treated with 250μg MCA at biweekly intervals for 6 treatments. Two weeks after the last treatment the animals will be treated with an immunosuppressive drug cyclophosphamide during the period when tumor development actually occurs. Control animals will be held without immunosuppression to compare the tumor latency period and the number of tumors per animal that develop. The immunosuppressive effect will be determined by tumor transplant or skin graft technique.

If this study shows a shorter latency it will be followed by studies using 1 or 2 MCA treatments followed by immune suppressive therapy.

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Source: https://www.industrydocuments.ucsf.edu/docs/xnyl0000

CTR-66

December 20, 1974

Objective:

To follow the immunocompetence of C3H/f mice treated by intratracheal instillation of MCA in CTR-49.

Procedure:

C3H/f mice will be given 6 or more IT treatments with 250μg MCA at biweekly intervals as described in CTR-49 for histological studies and transplant studies to parallel studies in CTR-6 by Dr. Levy. The spleens and thymuses of these mice will be removed and the average weight and cell ratio determined. These cells will then be used for phytohaemagglutinin (PHA) and lipopolysaccharide (LPS) mitogen in vitro response determination. It is anticipated that multiple doses will induce suppression of cellular response which will probably recover after MCA treatment is stopped. There will probably occur a stimulation of response during the period when tumor growth occurs.

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Source: https://www.industrydocuments.ucsf.edu/docs/xnyl0000

SECTION F. Scientific Data Processing and Analysis

(M.J. Haven, M.S.)

1003536732

Source: https://www.industrydocuments.ucsf.edu/docs/xnyl0000

Section F. Scientific Data Processing and Analysis

During the month of October 1974, the entire Microbiological Experiment Management System was transferred from the NIH Computer Center to a commercial facility. In order to transfer and rebuild the Management System, more than 100 data files, software support programs, and the 10,000 records on the data base (which accounts for over 1/2 million fields of experiment information) had to be moved. This transfer was made after an extensive search for a commercial computer that would be compatible with the NIH system as far as software support, costs and file editing capabilities. The transfer necessitated the leasing of an in-house terminal to be used for daily maintenance operations on the data base and periodic updating of current experiments supported by the system. At this time, an average of 5 hours/day is spent on the terminal by MA personnel for the maintenance and expansion of the Experiment Management System.

During the next year, the Scientific Data Processing and Analysis section will be involved with increasing the size capacity for file storage. At present, we can only store information of 30 experiments at one time. We will expand this number to 99 concurrent experiments. Also we now have the capability to store a maximum of 40 observations on each animal for 8 different observation dates during the course of an experiment. We will expand this to 15 different observation dates - thus increasing our total observation number to 15 per animal. We anticipate adding other experiments to the data base within the next 6 months. We are involved in developing summary reports from the data recorded on the autopsy reports that has been entered into the computer. This will add a new dimension in the analysis of the data.

Increased program effort will be focused on the development of support routines for the inhalation experiments. We hope to use new methods and summarization programs in analysing these results. One such method will be the use of a computer generated plotting package. Measures to implement such up-to-date and time saving procedures has already begun. These computer generated graphic plots can be photographed and may accompany any research paper to be published. We also intend to begin the design of an information retrieval system. This fully automated facility will provide the user with a powerfull tool for searching the data base for facts on any experiment. One may thus obtain immediate, organized and complete information on a given subject. This system will be both a research facility and a practical business tool for gathering information at the user's office via a computer terminal on any item of information that has been entered onto the data base.

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Source: https://www.industrydocuments.ucsf.edu/docs/xnyl0000

IV BUDGET FOR CTR-14

JULY 1, 1975 - JUNE 30, 1976

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IV BUDGET

The budget has been presented this year based on individual budgets for each section within this contract. Each scientific investigator has two technicians and the necessary animal caretaker support. Two histology laboratory sections (Sections D and E) are included. The services of the slide preparation laboratory (D) will be phased out during the first quarter of the year and all slides will be made in Section E. Dr. B. Sass (Section D) will continue to service this contract as his experience is invaluable. The Data Processing and Analysis Section (F) has become a very valuable part of our contract making available analysis of our data in greater detail than possible by manual operations.

We feel the budget presented here represents the lowest possible level to present an integrated, yet diversified program which is outlined in the proposal. The inflationary pressures have been exceedingly great during the past year and the future appears to promise even greater stress in this area. We have included funds for equipment which has been shared by other contracts in the past and has been supplemented on a short term basis with equipment to be used in the Inhalation Laboratory the next contract year. We feel only by going to permanent cages can we continue to operate an animal facility at a reasonable price.

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Source: https://www.industrydocuments.ucsf.edu/docs/xnyl0000

BUDGET FOR CTR-14 (July 1, 1975 - June 30, 1976)
(MA 1800-2222)

		Section of Contract						
		A*	B	C	D	E	F	Total
A.	Direct Labor (Schedule A)	$ 40,144	$ 27,026	$ 35,215	$ 8,794	$ 11,644	$ 10,075	$132,898
B.	Overhead (115% of A)	46,166	31,080	40,497	10,113	13,391	11,586	152,833
C.	Other Direct Costs	22,000	18,000	14,000	1,000	3,000	10,000	68,000
D.	Travel	1,500	500	1,000	500	--	700	4,200
E.	Total (A-D)	109,810	76,606	90,712	20,407	28,035	32,361	357,931
F.	G & A (16%)	17,570	12,257	14,514	3,265	4,485	5,178	57,269
G.	Overtime Premium	200	200	200	--	--	--	600
H.	Total Cost	127,580	89,063	105,425	23,672	32,521	37,539	415,800
I.	Fixed Fee	14,174	9,895	11,713	3,628	3,613	4,171	46,194
J.	Total Before Equipment	$141,754	$ 98,958	$117,138	$ 26,300	$ 36,134	$ 41,710	$461,994
K.	Equipment (Schedule B)							60,800
L.	Total							$522,794

*A. C.E. Whitmire, Chemical Carcinogenesis
B. R.E. Kouri, AHH-Related Chemical Carcinogenesis
C. C.F. Demoise, Chemical Carcinogenesis
D. B. Sass, Histology at Walkersville during transition period
E. Histology in Bethesda Laboratory
F. M. Haven, Data Processing and Analysis

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Source: https://www.industrydocuments.ucsf.edu/docs/xnyl0000

R: REDACTED MATERIAL

SCHEDULE A - Direct Labor for CTR-14 (MA 2222)
(July 1, 1975 - June 30, 1976)

Section/Personnel	$/Hr.	% Time	Hours	$	$
A. Chemical Carcinogenesis					
C.E. Whitmire, Ph.D.	REDACTED	50	963	REDACTED	
R.M. Mason, Sr. Tech.		100	1,926		
P.G. Stein, Tech.		100	1,926		
A. Zuna, Anim. Care.		100	1,926		
			6,741		
		R		2,626	40,144
B. AHH-Related Chem. Carcinogenesis					
R.E. Kouri, Ph.D.	REDACTED	10	193	REDACTED	
T. Rude, Tech.		100	1,926		
E. Kiss, Tech.		100	1,926		
E. Prieto, Jr. Tech.		50	963		
			5,008		
		R		1,768	27,026
C. Chemical Carcinogenesis					
C.F. Demoise, Ph.D.	REDACTED	75	1,445	REDACTED	
M.D. Avery, Tech.		100	1,926		
K. Thorson, Tech.		100	1,926		
H. Lopez, Anim. Care.		100	1,926		
			7,223		
		R		2,304	35,215
D. Histopathology-Walkersville					
B. Sass, D.V.M.		25	482		
F. Dorset, Tech.	REDACTED	25	482	REDACTED	
B. Fouch, Tech.		25	482		
			1,446		
		R		575	8,794
E. Histology-Bethesda					
S.A. Gosnell, Super. Tech.	REDACTED	50	963	R	
Vacancy, Tech.		100	1,926		
			2,889		
		R		762	11,644
F. Data Processing and Analysis					
M. Haven, M.S.	REDACTED	10	196	REDACTED	
P. Gradwell, Data Tech.		55	1,059		
B. Ross, Key Punch Tech.		50	963		
			2,218		
		R		659	10,075
			25,525 hrs. (13.24 man years)		$132,898

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Source: https://www.industrydocuments.ucsf.edu/docs/xnyl0000

Schedule B: CTR-14 (July 1, 1975 - June 30, 1976)

During the past years of this contract, CTR has operated in a facility shared by an NCI contract and has made use of the equipment of that contract. Due to the size of this contract, it has been essential to provide separate facilities, therefore there exist a shortage of essential equipment for an efficient operation. During the past year the equipment destined for CTR-22 inhalation laboratory has been utilized for CTR-14. When the new facility for CTR-22 is completed, this equipment must be moved, thus leaving CTR-14 without essential equipment. The following list is submitted of essential items to maintain the status quo in our operation:

CO_2 incubator	$ 3,000
Ice machine	650
Nitrogen freezer with inventory system	6,000
Centrifuge	4,000
Balance	1,000
Rotary apparatus	350
2 water baths	800
Automatic watering on existing racks	15,000
Permanent animal cages	30,000
	$60,800

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Source: https://www.industrydocuments.ucsf.edu/docs/xnyl0000

CURRICULUM VITAE FOR NEW PERSONNEL

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Source: https://www.industrydocuments.ucsf.edu/docs/xnyl0000

R: REDACTED MATERIAL

CURRICULUM VITAE - MILES J. HAVEN

BIRTH: R

EDUCATION:

1972	M.S.	Computer Science University of Maryland, College Park
1967	B.S.	Computer Science University of Maryland, College Park

PROFESSIONAL AFFILIATIONS: REDACTED

PRESENT POSITION: September, 1974 - present REDACTED

POSITION DESCRIPTION:

REDACTED

PRIOR EXPERIENCE:

1971 - 1974	
1967 - 1971	REDACTED
1968 - present	

10035367740

Source: https://www.industrydocuments.ucsf.edu/docs/xnyl0000

PUBLICATIONS - MILES J. HAVEN

Glaser, E., and Haven, M. Band Pass Noise Stimulation of the Simulated Basilar Membrane. J. of Acoustical Society of America, October 1972.

Haven, M. Simulation and Mbdel Building. M.S. Thesis, University of Maryland, January 1972.

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Source: https://www.industrydocuments.ucsf.edu/docs/xnyl0000

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VI PUBLICATIONS

Source: https://www.industrydocuments.ucsf.edu/docs/xnyl0000

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Whitmire, C.E., Demoise, C.F., Kouri, R.E. The Role of the Host in the Development of In Vivo Models for Carcinogenesis Studies. In: Symposium on Experimental Respiratory Carcinogenesis and Bioassays (ed. J.F. PARKS and E. KARBE) Springer-Verlag, 1974, pages 20-47.

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Kouri, R.E., Demoise, C.F., Whitmire, C.E. The Significance of the Aryl Hydrocarbon Hydroxylase Enzyme Systems in the Selection of Model Systems for Respiratory Carcinogenesis. In: Symposium on Experimental Respiratory Carcinogenesis and Bioassays (ed. J.F. PARKS and E. KARBE) Springer-Verlag, 1974, pages 48-61.

Kouri, R.E., Ratrie III, H., Whitmire, C.E. Genetic Control of Susceptibility to 3-Methylcholanthrene-Induced Subcutaneous Sarcomas. Int. J. Cancer 13: 714-720, 1974.

Kouri, R.E., Kiefer, R., Zimmerman, E.M. Hydrocarbon-Metabolizing Activity of Various Mammalian Cells in Culture. In Vitro, Vol. 10, pages 18-25, 1974.

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Benedict, W.F., Rucker, N., Mark, C., Kouri, R.E. Correlation Between the Balance of Specific Chromosomes and the Expression of Malignancy in Hamster Cells. J. Natl. Cancer Inst. (in press) 1975.

Kouri, R.E., Rude, T.H., Thomas, P.E., Whitmire, C.E. Studies on Pulmonary Aryl Hydrocarbon Hydroxylase in Inbred Strains of Mice. (submitted) 1974.

Kouri, R.E., Kurtz, S.A., Price, P.J., Benedict, W.F. Studies on the ara-C-Induced Malignant Transformation of Hamster and Rat Cells in Culture. (submitted) 1974.

Kouri, R.E. Genetic Control of Susceptibility to Cancer Induced by 3-Methylcholanthrene (MCA). Proceedings of the XI International Cancer Congress, October 1974.

Benedict, W.F., Rucker, N., Faust, J. and Kouri, R.E. Malignant Transformation of Mouse Cells by Cigarette Smoke Condensate. Journal of the National Cancer Institute (in press) 1975.

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Source: https://www.industrydocuments.ucsf.edu/docs/xnyl0000